UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/986,381	11/08/2001	Steven S. Sommer	1954-360	5135

6449 7590 09/12/2005

ROTHWELL, FIGG, ERNST & MANBECK, P.C.
1425 K STREET, N.W.
SUITE 800
WASHINGTON, DC 20005

EXAMINER	
STRZELECKA, TERESA E	
ART UNIT	PAPER NUMBER
1637	

DATE MAILED: 09/12/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/986,381	SOMMER ET AL.	
	Examiner	Art Unit	
	Teresa E. Strzelecka	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 July 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,5,10-15,18-25 and 27* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,5,10-15,18-25 and 27* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

5.00

DETAILED ACTION

1. This office action is in response to an amendment filed July 12, 2005. Claims 1-26 were previously pending. Applicants amended claims 1, 5, 10-12, 15, 18, 19, and 25, cancelled claims 2-4, 6-9, 16, 17, and 26, and added new claim 27. Claims 1, 5, 10-15, 18-25 and 27 are pending and will be examined.

2. Applicants' amendments and claim cancellations overcame the following rejections: rejection of claims 11, 18 and 19 under 35 U.S.C. 112, second paragraph; rejection of claims 1, 5, 8-10 and 26 under 35 U.S.C. 102(b) as anticipated by Jonason et al.; rejection of claims 1, 5, 7-10 and 26 under 35 U.S.C. 102(b) as anticipated by Diamandis; rejection of claims 1, 2, 4 and 6 under 35 U.S.C. 102(a) as anticipated by Miyajima et al.; rejection of claim 11 under 35 U.S.C. 103(a) over Jonason et al. and Leutenegger et al.; rejection of claims 12 and 20 under 35 U.S.C. 103(a) over Jonason et al. and Klein; rejection of claim 13 under 35 U.S.C. 103(a) over Jonason et al. and Klein in view of Goldsworthy et al.; rejection of claim 14 under 35 U.S.C. 103(a) over Jonason et al. and Klein in view of Aghassi et al.; rejection of claim 15 under 35 U.S.C. 103(a) over Jonason et al. and Klein; rejection of claim 25 under 35 U.S.C. 103(a) over Jonason et al. and Murphy in view of Buck et al.; rejections of claims 2, 3 and 6 under 35 U.S.C. 103(a) over Diamandis and Hearslev et al.; rejection of claim 11 under 35 U.S.C. 103(a) over Diamandis and Leutenegger et al.; rejection of claim 12 under 35 U.S.C. 103(a) over Diamandis and Hearslev et al. in view of Klein; rejection of claim 13 under 35 U.S.C. 103(a) over Diamandis, Hearslev et al. and Klein in view of Goldsworthy et al.; rejection of claim 14 under 35 U.S.C. 103(a) over Diamandis, Hearslev et al. and Klein in view of Aghassi et al.; rejection of claim 15 under 35 U.S.C. 103(a) over Diamandis and Klein; rejection of claim 18 under 35 U.S.C. 103(a) over Diamandis and Leutenegger et al., further in view of Shamsher et al., Felix et al. and Buck et al.; rejection of claim 19 under 35 U.S.C. 103(a) over

Diamandis and Leutenegger et al., further in view of Shamsher et al. and Buck et al.; rejection of claims 21-24 under 35 U.S.C. 103(a) over Diamandis, Hearslev et al. and Klein, in view of Chang et al.; rejection of claim 25 under 35 U.S.C. 103(a) over Diamandis and Murphy in view of Buck et al.

3. Applicants arguments regarding previously made claim rejections are moot in view of new grounds for rejection.

Priority

4. Applicant's claim for domestic priority under 35 U.S.C. 119(e) is acknowledged. However, the provisional application upon which priority is claimed fails to provide adequate support under 35 U.S.C. 112 for claims 27, 11, 18, 19 and 25 of this application. The provisional application No. 60/246,582 does not disclose PCR products longer than 2kb. Therefore the priority date of these claims is the filing date of the instant application, November 8, 2001.

Claim Rejections - 35 USC § 112

5. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. Claims 27, 11, 18, 19 and 25 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The new claim 27 contains a limitation "to form a PCR product of at least 1 kb in size", which introduces new matter into the claim. There is no support in Applicants' disclosure for a PCR product longer than 2kb, which is the length which was obtained Applicants (see Fig. 2; page

17, paragraph [0039]). Therefore, the limitation of the length of PCR product exceeding 2 kb is not supported by Applicants' disclosure.

Claim Interpretation

7. Applicants did not define the term "missense mutation", therefore it is interpreted according to its usual meaning in the art, as a mutation that changes a codon for one amino acid into a codon for a different amino acid.

Claim Rejections - 35 USC § 102

8. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

9. Claims 1, 5, 10 and 12 are rejected under 35 U.S.C. 102(a) as being anticipated by Kaserer et al. (J. Pathol., vol. 190, pp. 450-456, March 2000), as evidenced by Soussi (Cancer Res., vol. 60, pp. 1777-1788, April 2000) and Thompson-Hehir et al. (Diagn. Mol. Pathol., vol. 9, pp. 110-119, June 2000).

Regarding claim 1, Kaserer et al. teach a method for determining the frequency or nature of PCR mutations in samples, the method comprising:

identifying a single somatic cell in said sample that contains p53 protein accumulated by missense mutations in exons 5 through 9 (Kaserer et al. teach identifying by immunohistochemistry single somatic cells which contain accumulated p53 protein in colorectal adenocarcinoma samples (page 451, paragraphs 1-3; Fig. 1). Kaserer et al. do not specifically teach that p53 is accumulated in the cells by missense mutations in exons 5-9. As evidenced by Soussi, p53 protein accumulates

in cells due to mutations which affect its stability, and that mostly missense mutations lead to p53 accumulation (page 1777, fourth paragraph; page 1778, fourth paragraph). Also, Kaserer et al. teach missense mutations in exons 5, 6 and 8 (Table 2), therefore they teach this limitation).),

amplifying by PCR a DNA molecule of said identified single somatic cell which is at least 1 kb in size and which spans exons 5 to 9 of p53 to produce a PCR product (Kaserer et al. teach amplification of a DNA molecule from a single cell identified by staining using primers for exons 4-9, therefore they teach amplification of DNA spanning exons 5-9 (page 451, paragraphs 6-12; Fig. 3). As evidenced by Thompson-Hehir et al., exons 4-9 span about 2700 bp (Figure 2, p. 114), therefore, by teaching amplification of exons 4-9 Kaserer et al. inherently teach amplification of DNA which is at least 1 kb in size).), and

determining the frequency or nature of mutations in said amplified DNA PCR product (Kaserer et al. teach determination of the nature and frequency of mutations in the amplified PCR products (page 452, last paragraph; page 453; Table 2; Table 4).

Regarding claim 5, Kaserer et al. teach identifying the cells by immunohistochemical staining (page 451, paragraphs 1-3; Fig. 1).

Regarding claim 10, Kaserer et al. teach amplifications of exons 4-9 (page 451, paragraphs 6-8). Kaserer et al. do not specifically teach that the amplified DNA fragment is at least 2 kb in size. As evidenced by Thompson-Hehir et al., exons 4-9 span about 2700 bp (Figure 2, p. 114), therefore, by teaching amplification of exons 4-9 Kaserer et al. inherently teach amplification of DNA which is at least 1 kb in size).

Regarding claim 12, Kaserer et al. teach microdissection of formalin-embedded tissues (page 451, paragraph 10).

10. Claims 1, 5 and 10 are rejected under 35 U.S.C. 102(b) as being anticipated by Ponten et al. (Mut. Res. Gen., vol. 382, pp. 45-55, 1997), as evidenced by Soussi (Cancer Res., vol. 60, pp. 1777-1788, April 2000) and Thompson-Hehir et al. (Diagn. Mol. Pathol., vol. 9, pp. 110-119, June 2000).

Regarding claim 1, Ponten et al. teach a method for determining the frequency or nature of PCR mutations in samples, the method comprising:

identifying a single somatic cell in said sample that contains p53 protein accumulated by missense mutations in exons 5 through 9 (Ponten et al. teach identifying by immunohistochemistry single somatic cells which contain accumulated p53 protein in basal cell cancer samples (page 46, paragraphs 4, 5; Fig. 3). Ponten et al. do not specifically teach that p53 is accumulated in the cells by missense mutations in exons 5-9. As evidenced by Soussi, p53 protein accumulates in cells due to mutations which affect its stability, and that mostly missense mutations lead to p53 accumulation (page 1777, fourth paragraph; page 1778, fourth paragraph). Also, Ponten et al. teach missense mutations in exons 7 and 8 (Fig. 2, 5; page 50, paragraphs 4-6), therefore they teach this limitation).),

amplifying by PCR a DNA molecule of said identified single somatic cell which is at least 1 kb in size and which spans exons 5 to 9 of p53 to produce a PCR product (Ponten et al. teach amplification of a DNA molecule from a single cell identified by staining using primers for exons 4-9, therefore they teach amplification of DNA spanning exons 5-9 (page 46, paragraph 6; Fig. 4). As evidenced by Thompson-Hehir et al., exons 4-9 span about 2700 bp (Figure 2, p. 114), therefore, by teaching amplification of exons 4-9 Ponten et al. inherently teach amplification of DNA which is at least 1 kb in size).), and

determining the frequency or nature of mutations in said amplified DNA PCR product (Ponten et al. teach determination of the nature and frequency of mutations in the amplified PCR products (page 47, second paragraph; Table 1; Fig. 2, 5; page 50, paragraphs 4-6).

Regarding claim 5, Ponten et al. teach identifying the cells by immunohistochemical staining (page 46, paragraphs 4, 5; Fig. 3).

Regarding claim 10, Ponten et al. teach amplifications of exons 4-9 (page 46, paragraph 6). Ponten et al. do not specifically teach that the amplified DNA fragment is at least 2 kb in size. As evidenced by Thompson-Hehir et al., exons 4-9 span about 2700 bp (Figure 2, p. 114), therefore, by teaching amplification of exons 4-9 Ponten et al. inherently teach amplification of DNA which is at least 1 kb in size).

Claim Rejections - 35 USC § 103

11. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

12. Claims 12, 15 and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ponten et al. (Mut. Res. Gen., vol. 382, pp. 45-55, 1997), as evidenced by Soussi (Cancer Res., vol. 60, pp. 1777-1788, April 2000) and Thompson-Hehir et al. (Diagn. Mol. Pathol., vol. 9, pp. 110-119, June 2000) and Klein et al. (WO 2000/17390; cited in the previous office action).

A) Regarding claim 1, Ponten et al. teach a method for determining the frequency or nature of PCR mutations in samples, the method comprising:

identifying a single somatic cell in said sample that contains p53 protein accumulated by missense mutations in exons 5 through 9 (Ponten et al. teach identifying by immunohistochemistry

single somatic cells which contain accumulated p53 protein in basal cell cancer samples (page 46, paragraphs 4, 5; Fig. 3). Ponten et al. do not specifically teach that p53 is accumulated in the cells by missense mutations in exons 5-9. As evidenced by Soussi, p53 protein accumulates in cells due to mutations which affect its stability, and that mostly missense mutations lead to p53 accumulation (page 1777, fourth paragraph; page 1778, fourth paragraph). Also, Ponten et al. teach missense mutations in exons 7 and 8 (Fig. 2, 5; page 50, paragraphs 4-6), therefore they teach this limitation).),

amplifying by PCR a DNA molecule of said identified single somatic cell which is at least 1 kb in size and which spans exons 5 to 9 of p53 to produce a PCR product (Ponten et al. teach amplification of a DNA molecule from a single cell identified by staining using primers for exons 4-9, therefore they teach amplification of DNA spanning exons 5-9 (page 46, paragraph 6; Fig. 4). As evidenced by Thompson-Hehir et al., exons 4-9 span about 2700 bp (Figure 2, p. 114), therefore, by teaching amplification of exons 4-9 Ponten et al. inherently teach amplification of DNA which is at least 1 kb in size).), and

determining the frequency or nature of mutations in said amplified DNA PCR product (Ponten et al. teach determination of the nature and frequency of mutations in the amplified PCR products (page 47, second paragraph; Table 1; Fig. 2, 5; page 50, paragraphs 4-6).

Regarding claim 20, Ponten et al. teach tissue sections from patients at a risk of developing skin cancer due to prolonged sunlight exposure (page 45; page 46, first and second paragraphs).

B) Ponten et al. teach detection of p53 from fixed single cells by microdissection and PCR amplification of DNA from a single cell, but they do not teach cells obtained from a paraffin-embedded tissue section or amplification of DNA using two different DNA polymerases.

C) Regarding claim 12, Klein et al. teach amplification of DNA from single cells, which were chemically fixed (page 9, second paragraph; page 13, second paragraph) and isolated by microdissection (page 9, third paragraph). Klein et al. teach amplification of exons 4-9 of p53 in order to determine the presence of p 53 mutations (page 28, third paragraph).

Regarding claim 15, Klein et al. teach amplification of exons 4-9 of p53 in order to determine the presence of p 53 mutations, with the amplification reaction containing two polymerases, Taq and Pwo (page 28, third paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art to have used two DNA polymerases of Klein et al. in the method of p53 mutation detection of Ponten et al. The motivation to do so, provided by Klein et al., would have been that using two polymerases avoided Taq polymerase errors during PCR (page 28, third paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art to have used amplification of fixed cells of Klein et al. in the method of p53 mutation detection of Ponten et al. The motivation to do so, provided by Klein et al., would have been, as stated by Klein:

"The inventive method described herein can be applied to DNA of different sources, such as solid tumor DNA isolated from frozen sections and/or cryosections and/or paraffin embedded, formalin fixed specimens. For decades these tissue sections have been stored mainly for histopathological diagnosis. Single cells or small samples, comprising a limited amount of cells, from histopathological tissue can be screened for specific genetic changes and compared with other areas from the same tissue that may exhibit distinctly different histopathological features or, for control purposes, with areas of apparently normal tissue. Global screening of copy number sequence changes within a tumor genome from archival tissue material could increase the knowledge about cytogenetic alterations in solid tumors significantly. A direct comparison of these cytogenetic data

with histological and histochemical results and clinical follow up data would become possible." (page 13, second paragraph).

13. Claim 13 is rejected under 35 U.S.C. 103(a) as being unpatentable over Ponten et al. (Mut. Res. Gen., vol. 382, pp. 45-55, 1997), as evidenced by Soussi (Cancer Res., vol. 60, pp. 1777-1788, April 2000) and Thompson-Hehir et al. (Diagn. Mol. Pathol., vol. 9, pp. 110-119, June 2000), and Klein et al. (WO 2000/17390; cited in the previous office action), as applied to claim 12 above, and further in view of Goldsworthy et al. (Mol. Carcinogen., vol. 25, p. 86-91, 1999; cited in the previous office action).

A) The teachings of Ponten et al. and Klein are described above. Ponten et al. teach detection of p53 cells from fixed cells, but they do not teach ethanol as a fixative. Klein et al. teach using single cells obtained by microdissection from chemically fixed material, but do not teach ethanol as a fixative.

B) Regarding claim 13, Goldsworthy et al. teach amplification of RNA from single cells obtained by laser microdissection (page 86, second paragraph) from preparations which were chemically fixed with different agents, such as 70% ethanol, 95% ethanol, 10% neutral buffered formalin, 3% paraformaldehyde or acetone (page 87, second and third paragraphs) before embedding in paraffin (page 87, fourth paragraph). The tissue sections were used to extract single cells and amplify RNA by RT-PCR (page 87, fifth and sixth paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art to have used ethanol of Goldsworthy et al. as a fixative for single cell amplification of Klein et al. in the method of p53 mutation detection of Ponten et al. The motivation to do so, provided by Goldsworthy et al., would have been that ethanol fixation provided the best morphology, microdissection and RNA extraction results for paraffin-embedded tissue (Table 1; Abstract).

14. Claim 14 is rejected under 35 U.S.C. 103(a) as being unpatentable over Ponten et al. (Mut. Res. Gen., vol. 382, pp. 45-55, 1997), as evidenced by Soussi (Cancer Res., vol. 60, pp. 1777-1788, April 2000) and Thompson-Hehir et al. (Diagn. Mol. Pathol., vol. 9, pp. 110-119, June 2000), and Klein et al. (WO 2000/17390; cited in the previous office action), as applied to claim 12 above, and further in view of Aghassi et al. (U.S. Patent No. 6,649,368 B1; cited in the previous office action).

A) The teachings of Ponten et al. and Klein et al. are described above. Ponten et al. teach immunohistochemical staining of tissue sections for p53, but they do not teach subjecting the tissue section to steam heating in the presence of EDTA.

B) Regarding claim 14, Aghassi et al. teach composition and method of treating tissue sections to enhance immunohistochemical staining (col. 2, lines 40-59). The tissue samples are placed in a solution and heated to 120 C for 10-15 minutes, and the solution contains EDTA as a tissue activating agent (col. 3, lines 19-35; col. 5, lines 61-67).

It would have been *prima facie* obvious to one of ordinary skill in the art to have used the heating of tissue sections of Aghassi et al. in the method of p53 mutation detection of Ponten et al. and Klein et al. The motivation to do so, provided by Aghassi et al., would have been, as stated by Aghassi et al.: "Another advantage of the present invention is that the sample may be used on paraffin-embedded tissues without removing the paraffin embedding medium prior to treatment. Yet another advantage of the present invention is to provide a non-toxic, biodegradable composition for pretreatment of slides. Yet another advantage of the present invention is that the composition preferably substantially simultaneously: (i) removes the embedding medium from the tissue; (ii) improves immunohistochemical staining of the tissue in comparison to tissue that has not been contacted with the composition; and (iii) substantially hydrates the tissue."

15. Claims 21-24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ponten et al. (Mut. Res. Gen., vol. 382, pp. 45-55, 1997), as evidenced by Soussi (Cancer Res., vol. 60, pp. 1777-1788, April 2000) and Thompson-Hehir et al. (Diagn. Mol. Pathol., vol. 9, pp. 110-119, June 2000), and Klein et al. (WO 2000/17390; cited in the previous office action), as applied to claim 12 above, and further in view of Chang et al. (Mol. Med. Today, vol. 6, pp. 358-364, September 2000; cited in the previous office action).

A) The teachings of Ponten et al. and Klein et al. are described above. Ponten et al. and Klein et al. teach tissue sections obtained from patients with cancer, but do not teach tissue sections obtained from patients receiving treatment for cancer condition, such as radiation therapy, drug treatment or gene therapy.

B) Chang et al. teach gene therapy for p53 and its role in sensitizing cancer cells to chemotherapy (= cytotoxic drug treatment) and radiation therapy (Abstract; Fig. 1; page 361).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have monitored patients undergoing chemotherapy, radiation therapy or gene therapy of Chang et al. for p53 mutations by the method of Ponten et al. and Klein et al. The motivation to do so, provided by Chang et al. would have been that tumor cells with mutant p53 were less responsive to chemotherapy (page 358, last paragraph) and radiation treatment (page 359, last paragraph; page 360, second, third and fifth paragraphs), whereas presence of wild-type p53 introduced by gene therapy sensitized the cancer cells to cytotoxic drugs and radiation) (page 361, first and second paragraphs)

16. Claims 11 and 27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ponten et al. (Mut. Res. Gen., vol. 382, pp. 45-55, 1997), as evidenced by Soussi (Cancer Res., vol. 60, pp. 1777-1788, April 2000) and Thompson-Hehir et al. (Diagn. Mol. Pathol., vol. 9, pp. 110-119, June

2000), Klein et al. (WO 2000/17390; cited in the previous office action) and Leutenegger et al. (Vet. Immunol. Immunopath., vol. 71, pp. 291-305, 1999; cited in the previous office action).

Regarding claim 27, Ponten et al. teach a method for determining the frequency or nature of PCR mutations in samples, the method comprising:

(a) providing a paraffin-embedded tissue section containing human cells (Ponten et al. teach providing tissue sections containing human cells (Abstract; page 46, fourth paragraph).);

(b) identifying a single human somatic cell in said tissue section that contains p53 protein accumulated in said single somatic cell by missense mutations in exons 5 through 9 by immunohistochemical staining for p53 (Ponten et al. teach identifying by immunohistochemistry single somatic cells which contain accumulated p53 protein in basal cell cancer samples (page 46, paragraphs 4, 5; Fig. 3). Ponten et al. do not specifically teach that p53 is accumulated in the cells by missense mutations in exons 5-9. As evidenced by Soussi, p53 protein accumulates in cells due to mutations which affect its stability, and that mostly missense mutations lead to p53 accumulation (page 1777, fourth paragraph; page 1778, fourth paragraph). Also, Ponten et al. teach missense mutations in exons 7 and 8 (Fig. 2, 5; page 50, paragraphs 4-6), therefore they teach this limitation).);

(c) microdissecting said identified single human somatic cell from said tissue section (Ponten et al. teach microdissecting single cells from the tissue sections (page 46, fifth paragraph; Fig.3).);

(d) amplifying by PCR a p53 DNA sequence of said identified single somatic cell in the presence of carrier DNA using two different polymerases, wherein said DNA sequence is at least 1 kb in size and spans exons 5 to 9 of p53, to form a PCR product of at least 1 kb in size (Ponten et al. teach amplification of a DNA molecule from a single cell identified by staining using primers for

exons 4-9, therefore they teach amplification of DNA spanning exons 5-9 (page 46, paragraph 6; Fig. 4). As evidenced by Thompson-Hehir et al., exons 4-9 span about 2700 bp (Figure 2, p. 114), therefore, by teaching amplification of exons 4-9 Ponten et al. inherently teach amplification of DNA which is at least 1 kb in size).); and

(e) determining the frequency or nature of mutations in said PCR product by sequence analysis of said PCR product (Ponten et al. teach determination of the nature and frequency of mutations in the amplified PCR products (page 47, second paragraph; Table 1; Fig. 2, 5; page 50, paragraphs 4-6).

B) Ponten et al. teach detection of p53 from fixed single cells by microdissection and PCR amplification of DNA from a single cell, but they do not teach cells obtained from a paraffin-embedded tissue section, amplification of DNA using two different DNA polymerases or PCR products longer than 1 kb.

C) Regarding claim 27, Klein et al. teach amplification of DNA from single cells, which were chemically fixed (page 9, second paragraph; page 13, second paragraph) and isolated by microdissection (page 9, third paragraph). Klein et al. teach amplification of exons 4-9 of p53 in order to determine the presence of p 53 mutations (page 28, third paragraph). Klein et al. teach amplification of exons 4-9 of p53 in order to determine the presence of p 53 mutations, with the amplification reaction containing two polymerases, Taq and Pwo, and amplification products of 1374 bp and 1032 bp, and the presence of missense mutations in p53 (page 28, third paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art to have used two DNA polymerases of Klein et al. in the method of p53 mutation detection of Ponten et al. The motivation to do so, provided by Klein et al., would have been that using two polymerases avoided Taq polymerase errors during PCR (page 28, third paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art to have used amplification of fixed cells of Klein et al. in the method of p53 mutation detection of Ponten et al. The motivation to do so, provided by Klein et al., would have been, as stated by Klein:

> "The inventive method described herein can be applied to DNA of different sources, such as solid tumor DNA isolated from frozen sections and/or cryosections and/or paraffin embedded, formalin fixed specimens. For decades these tissue sections have been stored mainly for histopathological diagnosis. Single cells or small samples, comprising a limited amount of cells, from histopathological tissue can be screened for specific genetic changes and compared with other areas from the same tissue that may exhibit distinctly different histopathological features or, for control purposes, with areas of apparently normal tissue. Global screening of copy number sequence changes within a tumor genome from archival tissue material could increase the knowledge about cytogenetic alterations in solid tumors significantly. A direct comparison of these cytogenetic data with histological and histochemical results and clinical follow up data would become possible." (page 13, second paragraph).

D) Neither Ponten et al. nor Klein et al. teach carrier DNA.

E) Leutenegger et al. teach quantitative amplification of GADPH cDNA using calf thymus DNA as carrier DNA (page 298, second paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used a carrier DNA (which can be any DNA) of Leutenegger et al. in the p53 amplification method of Ponten et al. and Klein et al. The motivation to do so, provided by Leutenegger et al., would have been that using carrier DNA prevented low amounts of target DNA from adsorption to reaction tube walls (page 298, second paragraph).

17. Claim 18 is rejected under 35 U.S.C. 103(a) as being unpatentable over Ponten et al. (Mut. Res. Gen., vol. 382, pp. 45-55, 1997), as evidenced by Soussi (Cancer Res., vol. 60, pp. 1777-1788, April 2000), Thompson-Hehir et al. (Diagn. Mol. Pathol., vol. 9, pp. 110-119, June 2000) and Berg et al. (Clin. Chem., vol. 41, pp. 1461-66, 1995), Klein et al. (WO 2000/17390; cited in the previous office action) and Leutenegger et al. (Vet. Immunol. Immunopath., vol. 71, pp. 291-305, 1999; cited in the previous office action), as applied to claim 27 above, and further in view of Shamsher et al. (Gene, vol. 176, pp. 259-262, 1996; cited in the previous office action), Accession No. X54156 (June 1997; cited in the previous office action) and Buck et al. (Biotechniques, vol. 27, pp. 528-536, 1999; cited in the previous office action).

A) Ponten et al. teach PCR amplification of exons 4-9 using primers listed by Berg et al. in Table 1, but they do not teach specific primers with SEQ ID NO: 1 and 2. Forward primers for exon 5 amplification of Ponten et al. map to bp 12952-12967 and 13007-13025 of the p53 sequence, and the reverse primer for exon 9 amplification to bp 14777-14796 and 14891-14906 of the p53 sequence (see Table 1 of Berg et al.).

B) Shamsher et al. teach a 799 bp fragment of intron 4 of p53 and amplification of intron 4 with primers both outside and within the intron (Fig. 1). SEQ ID NO: 1 is 100% identical to bp 707-736 of the Shamsher et al. sequence (see sequence alignment). Further, SEQ ID NO: 1 maps to nucleotides 12983-13012 of the accession No. X54156 (see sequence alignment). Therefore, SEQ ID NO: 1 also overlaps with primer sequences for exon 5 amplification of Ponten et al.

C) SEQ ID NO: 2 is 100% identical to bp 14833-14862 of the sequence of p53 nucleic acid given by accession No. X54156 (see sequence alignment). Therefore, SEQ ID NO: 2 lies within primer sequences for exon 9 amplification of Ponten et al.

D) Thompson-Hehir et al. teach amplification primers for all of the exons of p53 (Fig. 2; Table 2). For example, a forward primer for exon 5 (5AF) amplification starts at bp 12989 and ends at position 13010 (within SEQ ID NO: 1), and a reverse primer for amplification of exon 9 (9R) starts at position 14843 and ends at position 14865, overlapping with SEQ ID NO: 2.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the method of Ponten et al. and Klein et al. with the use of functionally equivalent primers derived from the sequences of Shamsher et al. and a sequence with accession No. X54156, since Ponten et al. and Thompson-Hehir et al. teach primers for amplification of p53 sequences, and since Shamsher et al. and a sequence with accession No. X54156 provide sequences from which to chose such primers.

In the recent court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995), the Court of Appeals for the Federal Circuit determined that the existence of a general method of identifying a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologs, however, the Court stated,

> "Normally, a *prima facie* case of obviousness is based upon structural similiarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties (see page 9, paragraph 4 of attached ref)."

Since the claimed primers simply represent structural homologs selected from sequences of Shamsher et al. and a sequence with accession No. X54156, and in view of the fact that Ponten et al., Thompson-Hehir et al. and Shamsher et al. teach primers for p53 sequence amplification in order to determine p53 mutations, and concerning which a biochemist of ordinary skill would attempt to obtain alternate compounds with improved properties, the claimed primers and probes are *prima facie* obvious over the cited references in the absence of secondary considerations.

Buck expressly provides evidence of the equivalence of primers. Specifically, Buck invited primer submissions from a number of labs (39) (page 532, column 3), with 69 different primers being submitted (see page 530, column 1). Buck also tested 95 primers spaced at 3 nucleotide intervals along the entire sequence at issue, thereby testing more than 1/3 of all possible 18 mer primers on the 300 base pair sequence (see page 530, column 1). When Buck tested each of the primers selected by the methods of the different labs, Buck found that EVERY SINGLE PRIMER worked (see page 533, column 1). Only one primer ever failed, No. 8, and that primer functioned when repeated. Further, EVERY SINGLE CONTROL PRIMER functioned as well (see page 533, column 1). Buck expressly states "The results of the empirical sequencing analysis were surprising in that nearly all of the primers yielded data of extremely high quality (page 535, column 2)." Therefore, Buck provides direct evidence that all primers would be expected to function, and in particular, all primers selected according to the ordinary criteria, however different, used by 39 different laboratories. It is particularly striking that all 95 control primers functioned, which represent 1/3 of all possible primers in the target region. This clearly shows that every primer would have a reasonable expectation of success.

18. Claim 19 is rejected under 35 U.S.C. 103(a) as being unpatentable over Ponten et al. (Mut. Res. Gen., vol. 382, pp. 45-55, 1997), as evidenced by Soussi (Cancer Res., vol. 60, pp. 1777-1788, April 2000), Thompson-Hehir et al. (Diagn. Mol. Pathol., vol. 9, pp. 110-119, June 2000) and Berg et al. (Clin. Chem., vol. 41, pp. 1461-66, 1995), Klein et al. (WO 2000/17390; cited in the previous office action) and Leutenegger et al. (Vet. Immunol. Immunopath., vol. 71, pp. 291-305, 1999; cited in the previous office action), as applied to claim 27 above, and further in view of Shamsher et al. (Gene, vol. 176, pp. 259-262, 1996 ; cited in the previous office action), Felix et al. (J. Clin. Invest., vol. 89, pp. 640-647, 1992; cited in the previous office action) and Buck et al. (Biotechniques, vol. 27, pp. 528-536, 1999 ; cited in the previous office action).

A) Ponten et al. teach PCR amplification of exons 4-9 using primers listed by Berg et al. in Table 1, but they do not teach specific primers with SEQ ID NO: 1 and 3. Forward primers for exon 5 amplification of Ponten et al. map to bp 12952-12967 and 13007-13025 of the p53 sequence, and the reverse primer for exon 9 amplification to bp 14777-14796 and 14891-14906 of the p53 sequence (see Table 1 of Berg et al.).

B) Shamsher et al. teach a 799 bp fragment of intron 4 of p53 and amplification of intron 4 with primers both outside and within the intron (Fig. 1). SEQ ID NO: 1 is 100% identical to bp 707-736 of the Shamsher et al. sequence (see sequence alignment). Further, SEQ ID NO: 1 maps to nucleotides 12983-13012 of the accession No. X54156 (see sequence alignment). Therefore, SEQ ID NO: 1 also overlaps with primer sequences for exon 5 amplification of Ponten et al.

C) Felix et al. teach a 133 bp insertion within intron 9 (Fig. 2) and amplification of introns 5, 6, 7 and 8 (Fig. 1). SEQ ID NO: 3 is 100% identical to bp 53-85 of the 133 bp fragment (see sequence alignment).

D) Thompson-Hehir et al. teach amplification primers for all of the exons of p53 (Fig. 2; Table 2). For example, a forward primer for exon 5 (5AF) amplification starts at bp 12989 and ends at position 13010 (within SEQ ID NO: 1).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the method of Ponten et al. and Klein et al. with the use of a functionally equivalent primers of derived from the sequences of Shamsher et al. and Felix et al., since Ponten et al. Thompson-Hehir et al. expressly teach primers for amplification of p53 sequences, and since Shamsher et al. and Felix et al. provide sequences of intron 4 and insertion of exon 9. In particular, since Ponten et al. Thompson-Hehir et al. teach detection of mutations in p53,

using a primer complementary to the sequence of Felix et al. would have provided a detection of exon 9 insertion which might cause predisposition to acute lymphoblastic leukemia.

In the recent court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995), the Court of Appeals for the Federal Circuit determined that the existence of a general method of identifying a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologs, however, the Court stated,

> "Normally, a *prima facie* case of obviousness is based upon structural similiarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties (see page 9, paragraph 4 of attached ref)."

Since the claimed primers simply represent structural homologs selected from sequences of Shamsher et al. and Felix et al., and in view of the fact that Ponten et al. Thompson-Hehir et al., Shamsher et al. and Felix et al. teach primers for p53 sequence amplification in order to determine p53 mutations, and concerning which a biochemist of ordinary skill would attempt to obtain alternate compounds with improved properties, the claimed primers and probes are *prima facie* obvious over the cited references in the absence of secondary considerations.

Buck expressly provides evidence of the equivalence of primers. Specifically, Buck invited primer submissions from a number of labs (39) (page 532, column 3), with 69 different primers being submitted (see page 530, column 1). Buck also tested 95 primers spaced at 3 nucleotide intervals along the entire sequence at issue, thereby testing more than 1/3 of all possible 18 mer primers on the 300 base pair sequence (see page 530, column 1). When Buck tested each of the primers selected by the methods of the different labs, Buck found that EVERY SINGLE PRIMER worked (see page 533, column 1). Only one primer ever failed, No. 8, and that primer functioned when repeated. Further, EVERY SINGLE CONTROL PRIMER functioned as well (see page 533, column 1). Buck expressly states "The results of the empirical sequencing analysis were surprising

in that nearly all of the primers yielded data of extremely high quality (page 535, column 2)." Therefore, Buck provides direct evidence that all primers would be expected to function, and in particular, all primers selected according to the ordinary criteria, however different, used by 39 different laboratories. It is particularly striking that all 95 control primers functioned, which represent 1/3 of all possible primers in the target region. This clearly shows that every primer would have a reasonable expectation of success.

19. Claim 25 is rejected under 35 U.S.C. 103(a) as being unpatentable over Ponten et al. (Mut. Res. Gen., vol. 382, pp. 45-55, 1997), as evidenced by Soussi (Cancer Res., vol. 60, pp. 1777-1788, April 2000), Thompson-Hehir et al. (Diagn. Mol. Pathol., vol. 9, pp. 110-119, June 2000) and Berg et al. (Clin. Chem., vol. 41, pp. 1461-66, 1995), Klein et al. (WO 2000/17390; cited in the previous office action) and Leutenegger et al. (Vet. Immunol. Immunopath., vol. 71, pp. 291-305, 1999; cited in the previous office action), as applied to claim 27 above, and further in view of Murphy (WO 99/06598; cited in the previous office action) and Buck et al. (Biotechniques, vol. 27, pp. 528-536, 1999 ; cited in the previous office action).

A) Ponten et al. teach PCR amplification of sequencing of exons 4-9 using primers listed by Berg et al. in Table 1, but they do not teach specific primers with SEQ ID NO: 5-11. Forward primers for exon 5 amplification of Ponten et al. map to bp 12952-12967 and 13007-13025 of the p53 sequence (see Table 1 of Berg et al.) Further, SEQ ID NO: 5 maps to nucleotides 13015-13038 of the accession No. X54156 (see sequence alignment). Therefore, SEQ ID NO: 5 also overlaps with the second primer sequence for exon 5 amplification of Ponten et al.

C) Thompson-Hehir et al. teach amplification primers for all of the exons of p53 (Fig. 2; Table 2). For example, a forward primer for exon 5 (5AF) amplification starts at bp 12989 and ends at position 13010 of the p53 gene.

D) Murphy teaches a primer for amplification of exon 6 of p53, which is 100% identical to a primer with SEQ ID NO: 5 over bases 5-28, but has four more nucleotides (page 45, last paragraph; page 46, primer 5F (see sequence alignment).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the method of Ponten et al. and Klein et al. with the use of a functionally equivalent primers of Murphy and Thompson-Hehir et al., since Ponten et al. expressly teach primers for amplification of p53 exons, including exon 6, and since Murphy provides such a primer.

In the recent court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995), the Court of Appeals for the Federal Circuit determined that the existence of a general method of identifying a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologs, however, the Court stated,

"Normally, a *prima facie* case of obviousness is based upon structural similiarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties (see page 9, paragraph 4 of attached ref)."

Since the claimed primer simply represent a structural homolog of a primer of Murphy, being only 4 bp shorter and otherwise identical, and in view of the fact that Ponten et al., Thompson-Hehir et al. and Murphy teach primers for p53 exon amplification in order to determine p53 mutations, and concerning which a biochemist of ordinary skill would attempt to obtain alternate compounds with improved properties, the claimed primers and probes are *prima facie* obvious over the cited references in the absence of secondary considerations.

Buck expressly provides evidence of the equivalence of primers. Specifically, Buck invited primer submissions from a number of labs (39) (page 532, column 3), with 69 different primers

being submitted (see page 530, column 1). Buck also tested 95 primers spaced at 3 nucleotide intervals along the entire sequence at issue, thereby testing more than 1/3 of all possible 18 mer primers on the 300 base pair sequence (see page 530, column 1). When Buck tested each of the primers selected by the methods of the different labs, Buck found that EVERY SINGLE PRIMER worked (see page 533, column 1). Only one primer ever failed, No. 8, and that primer functioned when repeated. Further, EVERY SINGLE CONTROL PRIMER functioned as well (see page 533, column 1). Buck expressly states "The results of the empirical sequencing analysis were surprising in that nearly all of the primers yielded data of extremely high quality (page 535, column 2)." Therefore, Buck provides direct evidence that all primers would be expected to function, and in particular, all primers selected according to the ordinary criteria, however different, used by 39 different laboratories. It is particularly striking that all 95 control primers functioned, which represent 1/3 of all possible primers in the target region. This clearly shows that every primer would have a reasonable expectation of success.

20. No claims are allowed.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Teresa E. Strzelecka whose telephone number is (571) 272-0789. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished

applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

August 30, 2005

TERESA STRZELECKA
PATENT EXAMINER
Teresa Strzelecka